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Authors: Liutkus, Matthew, Robinson, Shawn, MacDonald, Bruce, and Reid, Gregor

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## QUANTIFYING THE EFFECTS OF DIET AND MUSSEL SIZE ON THE BIOPHYSICAL PROPERTIES OF THE BLUE MUSSEL, *MYTILUS* SPP., FECES EGESTED UNDER SIMULATED IMTA CONDITIONS

MATTHEW LIUTKUS,<sup>1</sup> SHAWN ROBINSON,<sup>2</sup> BRUCE MACDONALD<sup>1\*</sup>  
AND GREGOR REID<sup>1</sup>

<sup>1</sup>Biology Department and Centre for Coastal Studies and Aquaculture, University of New Brunswick, 100 Tucker Road, PO Box 5050, Saint John, NB, E2L-4L5, Canada; <sup>2</sup>St. Andrews Biological Station, 531 Brandy Cove Road, St. Andrews, NB, E5B 2L9, Canada

**ABSTRACT** Three size classes of mussels (*Mytilus* spp.) (small, 26–35 mm; medium, 45–54 mm; and large, 65–74 mm) were exposed to 4 experimental diets consisting of mixed algae, diatom pastes, salmon feed “fines,” or salmon feces. Salmon culture by-product particles (feces and feed fines) were found to have minimal effect on the biophysical properties of mussel feces when compared with those from an algal-based diet. Differences in fecal morphology (feces widths) of mussel feces were found to be minimal in small mussel sizes, but became more significant as mussel shell length increased (45–74 mm). Furthermore, faeces from fish farm-based diets were found to be significantly narrower than algal based diets. Absorption efficiencies of the 4 different diets were 87%, 81%, 90%, and 86%, respectively. Regardless of diet, small mussels produced feces that dispersed as a function of settling velocity (small, 0.18 cm/sec; medium, 0.29 cm/sec; and large, 0.54 cm/sec (settling velocity of 50% of particles)) over much larger areas than those feces produced by larger mussels, suggesting that the influence of mussel culture on benthic loading of organic material around an aquaculture site will tend to increase over time as the mussel crop grows to maturity.

**KEY WORDS:** blue mussel, *Mytilus*, feces size, settling velocity, diet effects, absorption efficiency

### INTRODUCTION

The Integrated MultiTrophic Aquaculture (IMTA) project in the Bay of Fundy, Canada, is currently transitioning from a research and development scale to a commercial scale of production. Species that are currently integrated and farmed alongside Atlantic salmon (*Salmo salar*) include blue mussels (*Mytilus edulis* and *Mytilus trossulus*), which can retain smaller salmon feed fines and fecal particles from the water column that are in an acceptable particle size range; and kelp (*Saccharina latissima* and *Alaria esculenta*), which absorb dissolved nutrients such as nitrogen and phosphorus. The larger organic particles not captured by suspension feeders are anticipated to be captured by a benthic component of detritivores that are capable of assimilating them. The food supply for mussels cultured using IMTA techniques is supplemented with pulses of salmon farm by-product particles including salmon feed fines and feces (MacDonald et al. 2011). We know that mussels have the capability of absorbing these additional particles; however, there is scant information available on the dispersal characteristics of the biodeposits produced (Reid et al. 2009, Reid et al. 2010). To assess the sustainability of IMTA systems in open water, we need to have a better understanding of the efficiency of energy transfer from the water column to the benthos. The blue mussel is a sessile suspension-feeding bivalve and it plays a key role in connecting high-quality, pelagic, suspended particulate matter to the benthic ecosystem (Dame 1993, Giles & Pilditch 2004, Newell 2004, Callier et al. 2006, Cranford et al. 2007). Mussels filter ambient suspended matter from the water column, sort and process particles, and egest three different types of biodeposits (Dame 1993). Particles such as silt and, at times, excess cleared organic material are rejected before ingestion and expelled from the inhalant siphon in the form of

a conglomeration of loosely packaged particles, or pseudofeces. Desirable particles are ingested and further processed, and either expelled during the second sorting stage as intestinal feces or processed by the digestive gland and later egested as true mussel feces (Dame 1993, Giles & Pilditch 2004, Callier et al. 2006).

Previous studies have characterized the biophysical properties (morphology, organic content, and so forth) of biodeposits such as pseudofeces and feces produced under natural conditions by various mussel species to assess the potential impacts of mussel culture (e.g., Giles & Pilditch 2004, Callier et al. 2006). Because of the relative infancy of the open-water IMTA concept in the West, few studies have addressed the potential effects of the salmon farm diet supplement on these characteristics of mussel biodeposits. If the diet affects the specific density of the fecal pellet, then the settling velocities of the pellet will increase and the dispersal distance will decrease, resulting in higher organic loading rates around the aquaculture sites. In addition to the overall number of mussels in culture, a cohort of growing mussels will change significantly in size and number during the culture period, and mussel body size may also have an influence on the biophysical properties of feces. The objectives of this study were 2-fold: to compare the biophysical properties of mussel feces produced from salmon farm diets with those produced from algal-based diets in a laboratory setting and to determine whether the size of the mussels will influence characteristics of the feces. Characterizing mussel biodeposits to determine their settling rates and patterns of dispersal are, therefore, very important features in estimating the efficiency of IMTA systems in determining how and where to site the various biofilters to capture the particles.

### MATERIALS AND METHODS

#### *Mussel Collection and Acclimation*

A group of *Mytilus* (*M. edulis* and *M. trossulus*) species mussels were gathered from the intertidal shoreline at two locations:

\*Corresponding author. E-mail: bmacdon@unbsj.ca  
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Back Bay (45°02'37" N, 66°52'30" W) and Passamaquoddy Bay/St. Croix River (45°05'16" N, 67°04'52" W) in the Bay of Fundy, Canada (Fig. 1). Selected mussels of three size classes (small, 26–35 mm; medium, 45–54 mm; and large, 65–74 mm) were collected from each location, transported to the laboratory, and stored separately in flow-through seawater tanks. Seawater was pumped from the mouth of the St. Croix River from a depth of 25 m, passed through a sand filter, and delivered at ambient seasonal temperature. During this 14-day period, no food was added to the tanks, but there was a very low background concentration of total particulate matter from the tank in-flows (<0.05 mg/L). Groups of 13 mussels from the medium and large size classes, and 25 mussels from the small size class, were selected randomly from each of the two locations (small,  $n = 50$ ; medium,  $n = 26$ ; and large,  $n = 26$ ). Mussels were then cleaned of any settled organisms and then numbered by gluing water-resistant paper numbers to the posterior end of either the dorsal or ventral side of the mussel shell.

#### Experimental Design and Diet Characteristics

Mussels from each of the 3 size classes were exposed to 4 diet mixtures (2 algal-based and 2 salmon farm-based diets). The mixed algal diet consisted of concentrated *Phaeodactylum tricornutum*, *Chaetocerus-B*, and *Nanochloropsis oculatata* (5–15  $\mu$ m, typical maximal diameter), whereas the diatom diet was composed of a concentrated paste of a monoculture of *Thalassiosira weissflogii* (15–25  $\mu$ m, typical maximal diameter; Table 1). The

salmon farm diets—fines diet, was composed of a (Shur-Gain, Nutreco, Truro, N.S./Canada) extruded dry feed pellet ground, sieved, and filtered (100  $\mu$ m), whereas the feces diet was composed of fecal deposits from second-year class Atlantic salmon (*S. salar*) fed the same feed used for the fines diet (Table 1). Salmon feces were collected by placing a sump container under the outflow pipe of a salmon holding tank in the laboratory, allowing for accumulation of salmon feces in the sump container; feces were recovered and frozen in a –20°C freezer using the technique adopted by Ogunkoya et al. (2006). Salmon based diets were blended, and all diets were passed through a 100- $\mu$ m filter prior to being diluted in the header tanks delivery system.

#### Experimental Setup

The experimental diet delivery system consisted of a 136-L header tank in which diet and seawater were first mixed. Diets for each trial were prepared in a 7-L bucket to create a “stock broth”. The broth was diluted serially when needed, and pumped at a constant rate by a peristaltic pump and further mixed and diluted in the header tank. Desired concentrations of experimental diets less than 5 mg/L were achieved by adjusting the speed settings of the peristaltic pump and the concentrations of the stock broth. Ambient seawater temperature varied seasonally, making it necessary to use a submerged heat exchange coil in the header tank to maintain a narrow temperature range when exposing the mussels to the diets ( $9 \pm 2^\circ\text{C}$ ). Diets and seawater were mixed and aerated in the header tank with 2 submerged air

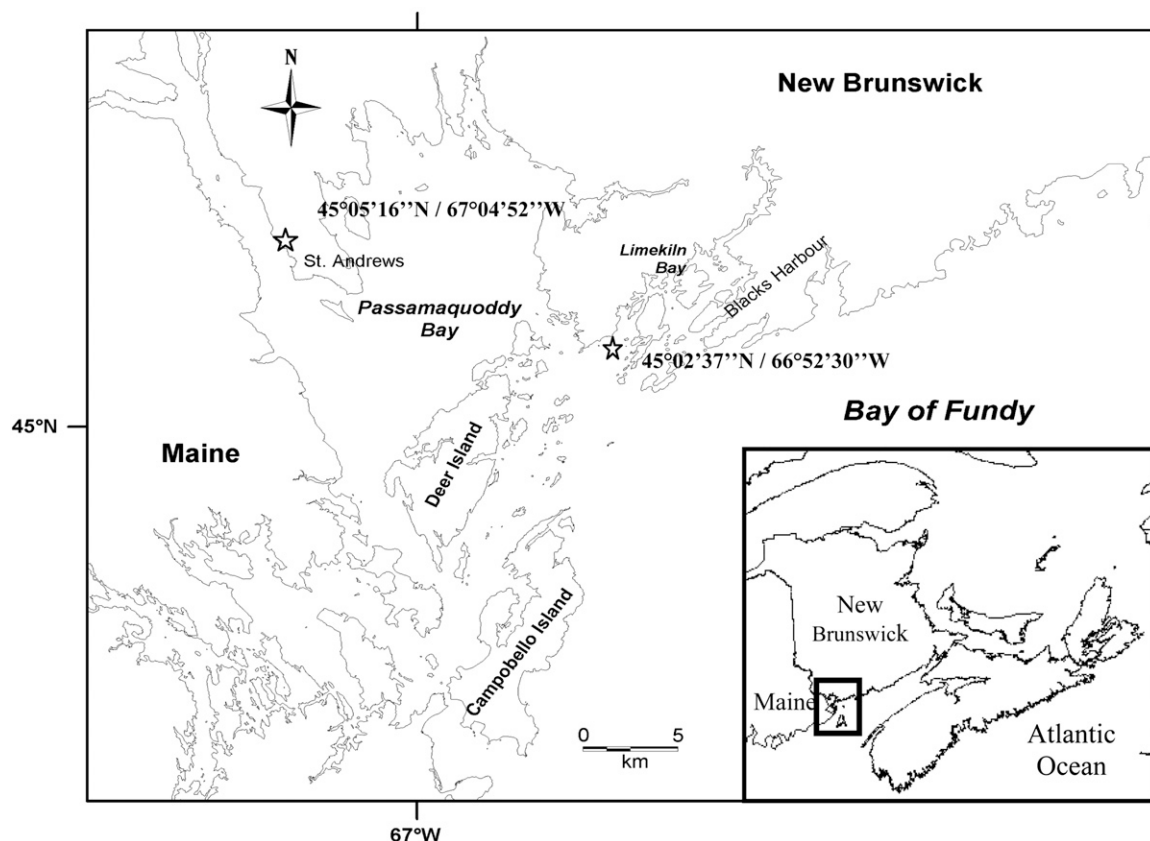


Figure 1. Collection sites for experimental mussels (☆) Back Bay (45°02'37" N, 66°52'30" W) and Passamaquoddy Bay/St. Croix River (45°05'16" N, 67°04'52" W).

**TABLE 1.**  
**Concentration (total particulate matter) and organic content of experimental diets: mixed algal, diatom, fines from feed, and salmon feces.**

	Mixed Algal Diet	Diatom Diet	Fines from Salmon Feed Diet	Salmon Feces Diet
TPM $\pm$ SD (mg/L)	4.1 $\pm$ 0.5	3.9 $\pm$ 0.4	3.8 $\pm$ 0.8	3.7 $\pm$ 0.9
OC $\pm$ SD (%)	76.9 $\pm$ 0.4	65.6 $\pm$ 0.8	93.1 $\pm$ 0.3	77.1 $\pm$ 2.2

Sample size for all diet concentration (total particulate matter; TPM) measurements ( $n = 27$ ) and diet organic content (OC; measured as a percentage) ( $n = 3$ ). Data describing diets are the mean  $\pm$  SD of 3 replicate water samples collected from the outflow valve of the control feeding chamber during 3 deposit trials ( $n = 9$ ). TPM was calculated as the dry weight of the water sample; OC was calculated by weight lost after combustion.

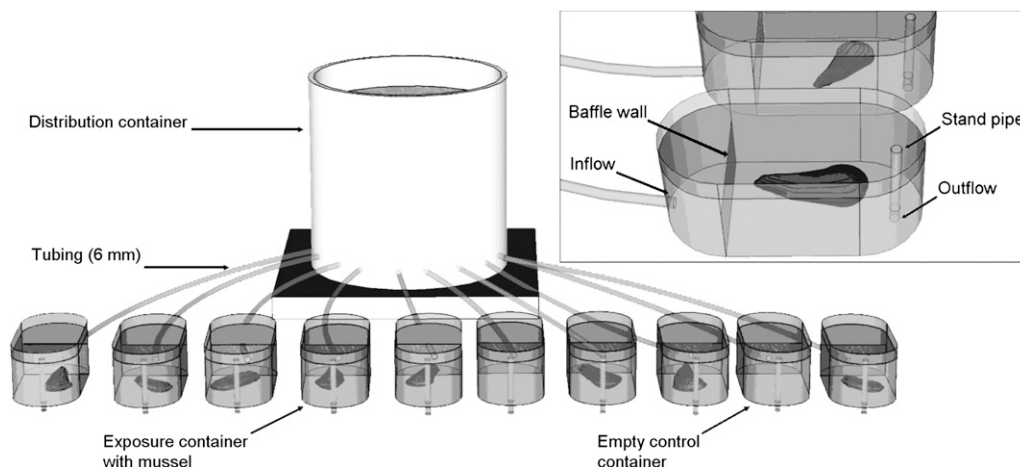
stones to keep particles suspended, and gravity was used to supply water to the distribution container. The header tank supplied a mixture of seawater and diet to the distribution container (40-cm height, 20 L) constructed with an overflow opening at a fixed height from the top that maintained a constant gravitational head pressure (Fig. 2). The distribution container also held submerged air stones to keep particles suspended. Water was distributed individually through 6-mm plastic tubing grouped inside the center of the distribution container delivered to each exposure chamber (length, 16 cm; width, 9 cm; height 12 cm; water volume, 1.05 L) at a constant flow rate of 290 mL/min. This rate was selected because flows of 200 mL/min and higher have been shown to prevent diet depletion in this type of exposure chamber, and allows the clearance rate to be independent of flow (Hawkins et al. 1998, Giles & Pilditch 2004, Hatton et al. 2005). Exposure chambers were designed with a baffle wall 4 cm away from the inflow pipe, providing micromixing in the chamber (Armsworthy et al. 2001, Hatton et al. 2005) (Fig. 2). Hatton et al. (2005) described the water current dynamics of a similar chamber, and how bottom turbulence is reduced by inserting baffles, thereby preventing biodeposit resuspension or microerosion. Water

exited the exposure chamber through an 8-cm-high stand pipe located at the opposite end of the exposure chamber.

Mussel biodeposits were collected during a series of randomized trials for both diet and mussel size to minimize any possible effects of time. During each trial, 8 randomly selected mussels were assigned to a random exposure chamber, and the 2 remaining exposure chambers were left empty to enable the characteristics of the particular diet to be determined. The number of mussels in each exposure chamber was determined by the size class being tested—1 mussel in each chamber for medium and large mussels, and 2 mussels per chamber for the small mussels. Exposure to diets commenced 4 h after the mussels were placed in the chambers, allowing them to acclimate to the experimental exposure chamber. Experimental start time  $T_0$  was designated at 2.5 h after first exposure to the experimental diet. Any biodeposits produced during the 2.5-h acclimation period were discarded, and collections continued for an additional 8 h. A large pipette was used to remove all biodeposits from each exposure chamber, and much care was taken not to break their form during handling periods. Biodeposit production experiments consisted of 4 diet exposures with 3 replicates of each diet for 3 size classes, for a total of 36 experimental deposit trials (4 diets  $\times$  3 sizes  $\times$  3 replicates).

#### *Biodeposit and Diet: Composition and Determination of Absorption Efficiency*

Biodeposits and samples of the diet were filtered onto “ashed” and preweighed 47-mm GF/C filters, rinsed with 10 mL distilled water, and placed immediately in a  $-80^\circ\text{C}$  freezer for later organic content (OC) analysis. The OC of the filtered biodeposit and diet were determined by removing filters from the  $-80^\circ\text{C}$  freezer, placing them in marked aluminum dishes, and drying at  $60^\circ\text{C}$  for 24 h (Giles & Pilditch 2004). Filters were removed and reweighed, and the weight was recorded as total particulate matter (measured in micrograms per liter) during that designated exposure period. Filters were then combusted at  $430^\circ\text{C}$  for 6 h, and the OC (measured as a percentage) of both biodeposits and diet were calculated as weight loss after combustion (Giles & Pilditch 2004). The absorption efficiency (AE;



**Figure 2.** System to deliver experimental diets simultaneously to individual mussels and empty control containers at constant flow rates. Inset shows details of baffle wall to ensure mixing, and standpipe to maintain constant volume. See text for details.



measured as a percentage) of experimental diets was calculated using the following equation:

$$AE (\%) = (\text{Diet} - \text{Feces}) / [(1 - \text{Feces})\text{Diet}] \times 100$$

where Diet describes the organic portion in the experimental diet and Feces describes the organic portion in biodeposits (Conover 1966).

#### *Morphology of Biodeposits*

Morphologies of individual biodeposits were measured by transfer by pipette into marked, individual white plastic holding dishes (7 mL). Biodeposits were photographed under a dissecting microscope, and digital pictures were later analyzed using image analysis software (Image Pro Plus, version VI, 4.5.1.2.2, Silver Spring, MD), and length, width, area, and aspect (ratio of length to width) were measured from the biodeposit images (Fig. 3).

#### *Settling Velocity of Biodeposits*

Settling velocities were assessed in a temperature-regulated double-tube settling column (height, 2.0 m; outer diameter, 0.32 m; inner diameter, 0.19 m). The column was supplied by the same seawater used in the exposure trial, and was regulated during the experimental period by pumping warm or ambient water through the outer column ( $11 \pm 1^\circ\text{C}$ ), thereby minimizing fluctuation in water temperature of the inner test column (Giles & Pilditch 2004). The settling column was filled 8 h prior to density trials, and a preliminary dye study showed no distinct upward eddies. Variation in water temperatures between top, middle, and bottom was found to be within  $\pm 1^\circ\text{C}$ , comparable with other studies (Hawkins et al. 1998, Giles & Pilditch 2004, Hatton et al. 2005). To measure settling velocity of individual fecal deposits, feces were introduced gently by pipette just below the water surface of the inner column. Determining the settling velocity timing did not begin until the biodeposit visually crossed the “starting line”—a line 0.35 m from the top of the column marked on a board behind the column. The purpose of the initial untimed settling distance was to negate potential acceleration effects resulting from inertial forces caused by introduction of the biodeposit. Biodeposits were timed over the duration for them to fall 0.90 m. Data from other published works showed no differences in settling velocity over three 0.30-m sections, when they used one overall measurement of 0.90 m (Giles & Pilditch 2004, Callier et al. 2006).

#### *Statistical Analysis*

Data sets were tested for normality and homogeneity of variance. If the assumptions were violated, logarithmic transformations were applied to enable the use of parametric tests. In cases

in which homogeneity of variance and normality could not be met, nonparametric tests were used. Two-way ANOVA was used to determine whether mussel size class and diet treatment had a significant influence on feces width and settling velocity. Tukey-Kramer multiple comparisons were used to determine whether significant differences existed between the means of feces width and settling velocity for the different treatment groups. To determine which measurements of biodeposit morphology had the strongest relationship with individual settling velocity, a best-fit regression analysis was performed.

Biodeposits from a single individual are unlikely to be independent; therefore, each biodeposit was treated as a statistical replicate for all analyses based on inferences by Giles and Pilditch (2004). They determined that, although biodeposits from a single size class can be assigned to the individual mussel that egested it, the variations in biodeposit morphology from a single mussel are greater than the variations between mussels. NCSS (Number Cruncher Statistical Software, Kaysville, UT) (Hintze 2004) was used for all statistical analyses.

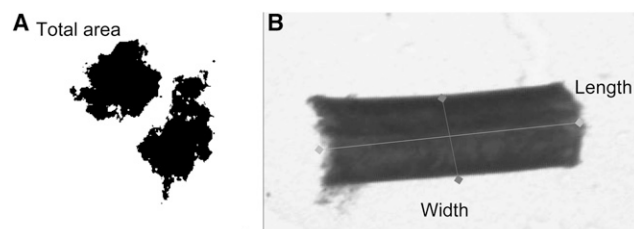
## RESULTS

#### *Experimental Observations*

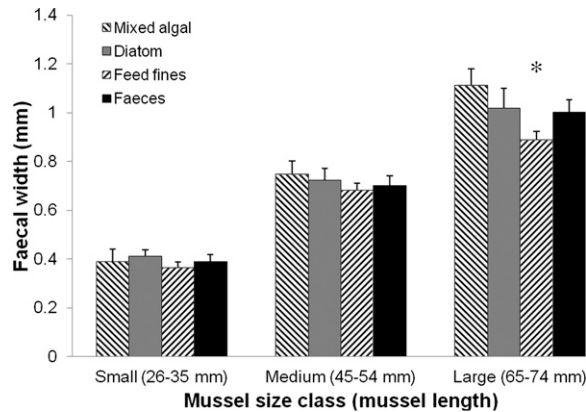
Pseudofeces production began within 15 min of exposure to the diets, and then slowed with the production of partially formed feces, followed by fully formed feces approximately 2.5–3.0 h after first exposure. Pseudofeces produced on the algal-based diets were amorphous in shape and were very fragile, yet still allowed for handling between measurements. Because of the fragility of pseudofeces produced on the salmon farm diets, their flocculent properties caused extensive fractures, and dispersion occurred even during gentle handling. Consequently, pseudofeces from all diets were not considered for further measurements or analysis. Egested feces had distinct colorations that were related to the specific coloration of the diet to which they were exposed. Feces produced on the mixed algal diet were dark green, the diatom diet produced light-brown feces, the salmon fines diet produced pale-yellow feces, and the salmon feces diet produced pale-brown feces.

#### *Feces Width*

Mussels produced feces that had a distinct partial cylindrical shape with a longitudinal groove down the center, similar in morphology to those found by Giles and Pilditch (2004). All diets produced a range of mean feces width between 0.36 mm and 1.11 mm, whereas the large mussels produced larger feces by a factor of 3 compared with small and medium mussels (Fig. 4). A general linear model was fitted to log-transformed biodeposit width data, with mussel size class and diet as factors; however, the resulting interaction term was significant ( $F = 33.95$ ,  $df = 6$ ,  $P = 0.04$ ). The model was broken down for individual factor analysis. A 1-way ANOVA of log-transformed feces width and mussel size class as an incorporated factor revealed significant differences (Table 2). Tukey tests revealed differences ( $P < 0.05$ ) across all 3 size classes in relation to feces width (Fig. 4). Further analysis of variance among each mussel size class revealed significant differences (Table 2) in feces width between the 4 diets. Tukey tests ( $P < 0.05$ , Fig. 4) indicate that differences in width of feces among diets were



**Figure 3.** Examples of mussel biodeposits and the measurements determined in the morphological analysis. (A) Pseudofeces. (B) Feces.



**Figure 4.** Mean feces width for 3 size classes of mussels exposed to 4 experimental diets. Error bars represent the SD of the data set. \*Significant difference ( $P < 0.05$ ) based on the multiple comparison test in each size class.

minimal in the small-mussel class, but become more pronounced as mussel size class increased. The mean width of feces produced on salmon farm-based diets appeared to be narrower than feces produced on algal-based diets for all 3 mussel size classes, but a significant difference was observed only for large mussels (Fig. 4).

#### Settling Velocity of Feces

We attempted to compare empirically derived and predicted mussel feces settling velocity (measured in centimeters per second) data, but calculations of settling velocities using Stokes' law could not be carried out because of the noncompliance of the assumption requiring a low Reynolds number (the ratio of inertial to viscous force)  $Re > 0.4$ . Empirically measured settling velocities of feces were generally similar in small size class, but became more variable in the medium and large size classes of mussels (Fig. 5). A general linear model was fitted to log-transformed feces settling velocity data with mussel size

class and diet as factors. Settlement velocities were a function of mussel size class and diet (Table 2), whereas the interaction term was not significant. A Tukey-Kramer multiple comparison test revealed that significant differences ( $P < 0.05$ ) among all 3 size classes were observed in relation to settling velocities. Overall, settling velocities were clearly different among mussel size classes, and they fell in a range of 0.2–0.3, 0.3–0.5, and 0.8–1.5 cm/sec for small, medium, and large mussels, respectively (Fig. 5, Table 2). Although there were no significant differences in settling velocity for feces produced on the mixed algal diets and the farm-based diets, feces produced on diatom diets settled significantly slower, at least for medium and large mussels (Fig. 5).

Correlation analyses of feces settling velocity and morphology measurements (width, length, and aspect) revealed that the strongest relationships existed between settling velocity and feces width for each diet group (algal,  $r = 0.86$ ; salmon farm,  $r = 0.82$ ; Pearson's correlation coefficient,  $P < 0.05$ ). Although, relationships among settling velocity, length, and aspect morphology measurements were also significant ( $P < 0.05$ ), they were not as well correlated with settling velocity as width (pooled; length,  $r \sim 0.60$ ; aspect morphology,  $r \sim 0.50$ ). The mathematical relationship between feces settling velocity and feces width was best explained by an exponential relationship, with high  $R^2$  values explaining 88–97% of the variance (Fig. 6), whereas a linear relationship produced  $R^2$  values that accounted for 71–85% of the variance.

#### Mussel Feces Organic Content and Absorption Efficiency

The OCs of experimental diets were within approximately 25% of each other, ranging from 65.9–93.1% (Table 3). The resulting OC of the feces ranged from 25.7–59.4%, within 34% of each other. A significant positive correlation between diet and the respective feces OC was observed ( $r = 0.94$ ), revealing that the OC of the diet is reflected in the OC of the feces (Table 3). Furthermore, the OC of the feces was significantly lower (Mann-Whitney  $U = 14.5$ ,  $n_1 = 27$ ,  $n_2 = 3$ ,  $P < 0.05$ ) than that of the corresponding diet, which indicated that mussels were capable of extracting OC from all diets tested. Corresponding mean estimates of AE for diets ranged from 81–90%, with values for salmon feed > mixed algae = salmon farm > diatom (Reid et al. 2010) (Table 3).

## DISCUSSION

#### Relationship Between Mussel Size and the Size of Feces

We found a strong relationship between increasing width of the feces and increasing mussel shell length, which confirms the observations by Callier et al. (2006) for the same species. Mussels produce a string of fecal matter that breaks into sections of variable length (depending on mussel orientation and the ambient flow rates). Based on these observations, it has been recommended that feces width be the indicator of mussel feces size (Giles & Pilditch 2004). These findings will assist in predicting the fecal dispersion characteristics of a cohort of growing mussels at an aquaculture site. The correlation of fecal pellet morphometrics with body size has been reported for various other species (Small et al. 1979, Giles & Pilditch 2004, Buryniuk et al. 2006, Callier et al. 2006). Small et al. (1979) reported differences in the volume of small and large copepod (*Anomalocera patersoni*)

**TABLE 2.**

**Analysis of variance reports examining the influence of mussel size class and diet on the feces width and sinking velocity of mussel feces produced.**

Source	df	F Ratio	P	Power	n
(A)					
Size class	2	5,477.7	<b>&lt;0.001</b>	1.00	743
(B)					
Diet	3	94.50	<b>&lt;0.001</b>	0.95	743
(C)					
Size class	2	3,978.4	<b>&lt;0.001</b>	1.00	743
Diet	3	47.1	<b>&lt;0.001</b>	1.00	743
Size class × diet	6	0.99	0.38	0.29	

Dependant variables were log transformed prior to analysis. (A) ANOVA results from  $\ln$  feces width (measured in millimeters) and mussel size class (measured in millimeters). (B) ANOVA results from  $\ln$  feces width (measured in millimeters) and diet. (C) Two-way ANOVA for  $\ln$  sinking velocity (measured in centimeters per second), mussel size class (measured in millimeters), and diet. Statistically significant values are in bold type.

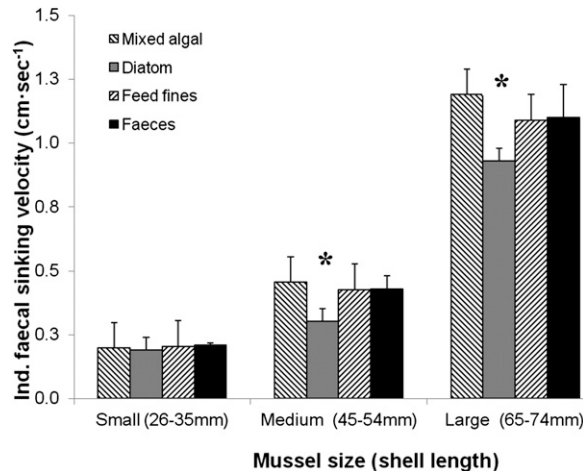


Figure 5. Mean sinking velocities for feces from the 3 size classes of mussels exposed to 4 experimental diets. Error bars represent the SD of the data set. \*Significant difference ( $P < 0.05$ ) based on the multiple comparison test in each size class.

fecal pellets, describing how fecal pellets produced by smaller copepods were of little consequence to the overall vertical organic flux, whereas larger copepods fecal pellets represented the majority of the vertical matter flux. Giles and Pilditch (2004) found green-lipped mussels (*Perna canaliculus*) to have an increasing feces size with increasing shell length. A similar effect has also been reported with salmon culture (Buryniuk et al. 2006), suggesting such size-based relationships extend logically to other culture species, as one might expect.

#### Effect of Diet on Biodeposit Biophysical Properties

Diet had an effect on the width of the fecal pellets produced across all mussel size classes. The finding that salmon farm-based particles producing narrower feces can be compared with observations by Giles and Pilditch (2004). They reported that *P. canaliculus* mussel feces width were, in general, narrower when silt particles were added to an experimental diet, compared with a natural diet (0.42–1.19 mm and 0.54–1.49 mm, respectively). The alteration in feces morphology resulting from a change in diet has also been observed in other species. Small et al. (1979) explained differences in copepod feces volume according to the type of algal species consumed. Copepods ingesting diatom species produced fecal pellets that were wider and more loosely packed compared with narrow feces produced from ingesting varying algal species (algae,  $1.5 \times 10^5 \mu\text{m}^3$ ; diatoms,  $12.3 \times 10^5 \mu\text{m}^3$ ) (Small et al. 1979).

Mussels are known to ingest particles from both algal and salmon farm diets, and diet was found to affect significantly the OC of the feces produced (Reid et al. 2010, MacDonald et al. 2011) (Table 3). These findings further confirm the important role of blue mussels in the IMTA setting as a biofilter for excess particulate material at salmon farms. Diet quality has been shown to influence AE, with relatively high values being observed for mussels in this study (80–90%) compared with many previous studies. Cranford and Hill (1999) reported a range of AE between 30% and more than 90%, whereas Bayne et al. (1989) reported AE values between 50% and 60% on experimental diets. AE has been reported to be largely a function of dietary OC. Hawkins et al. (1998) provide graphical representations of increases in AE until dietary OC reaches approximately 80%, at which point the relationship begins to plateau, ultimately becoming asymptotic.

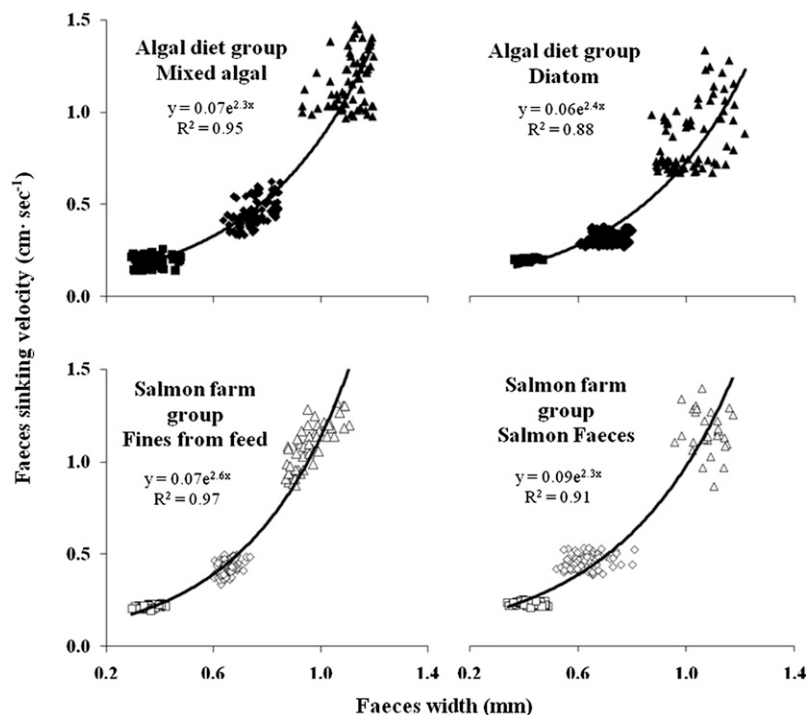


Figure 6. Relationships between sinking velocity of feces and corresponding width of feces for 3 size classes of mussels exposed to 4 experimental diets (filled symbols, algal diet; open symbols, salmon farm diet). Each diet fraction consists of 3 mussel size classes (mussel shell length): small, 26–35 mm (□); medium, 45–54 mm (◇); and large, 65–74 mm (Δ).



**TABLE 3.**  
**Organic content (%) of the 4 experimental diets and the feces produced from those diets, plus estimates of mussel absorption efficiency (AE; %).**

Origin	Diet			
	Algal		Salmon Farm	
	Mixed Algal	Diatom	Salmon Feed	Salmon Feces
Feces	29.4 ± 1.1	25.7 ± 1.0	59.4 ± 1.3	32.4 ± 0.7
Diet	76.9 ± 0.4	65.9 ± 0.8	93.1 ± 0.3	77.1 ± 2.2
AE	87	81	90	86

Sample sizes for measurements of organic content of feces ( $n = 27$ ) and diet ( $n = 3$ ) per experimental diet.

Data are the combination of all 3 size classes, and are the means ( $\pm$ SE) of the grouped data set. Organic content was derived from the weight lost after combustion. AE is the efficiency with which organic material is absorbed from ingested food material (derived from Conover (1966)).

Results from the current study indicate that AE values for salmon farm-based particulates were higher than those for commercial-based algal diets. This suggests that progressive stripping of organic material from salmon farm particles can occur with high efficiency. These findings confirm reports of augmented growth rates of mussels grown adjacent to salmon farms compared with reference sites (Lander et al. 2004). The OC of salmon farm-based mussel feces were still relatively high (25% and 50%). Thus, feces settling under mussel rafts co-cultured alongside salmon pens may still have sufficient potential as a resource for further integrated culture on a benthic–trophic level.

We observed that feces produced from the diatom diet settled more slowly than those produced from all other diets. This finding is in contrast to observations by Small et al. (1979), who observed that copepods that fed on diatoms and silt produced feces that settled more quickly (100.7 m/day) than feces produced when copepods were fed silt and other nondiatom species of phytoplankton (28 m/day). Giles and Pilditch (2004) also found changes in settling velocity according to diet and two size classes, with the addition of silt increasing the settling rate (without silt, 0.2 cm/sec and 1.6 cm/sec; with silt, 0.95 cm/sec and 4.5 cm/sec). Obviously, the composition of the local phytoplankton community in the Bay of Fundy, including the spring diatom bloom and the summer dinoflagellate blooms, will have a significant influence on the dispersal patterns of mussel feces from IMTA sites.

#### Settlement Velocities of Mussel Feces

The size of mussels was shown to have a strong relationship to settling velocities of feces. Settling velocities and density ranges measured in our study (0.17–1.6 cm/sec and 1.027–1.037 g/cm<sup>3</sup>, respectively) fall in lower ranges observed in other studies of *M. edulis* feces (Chamberlain et al. 2001, Callier et al. 2006). Slower settling velocities observed in our study could be explained by the absence of dense inorganic particles (such as silt) in our experimental diets (Chamberlain et al. 2001, Giles & Pilditch 2004). Although silt particles are a common and variable component of the suspended particulate matter of coastal marine waters, we tried to differentiate experimental diets without the incorporation of additional silt particles other than what

was available in the ambient seawater used to make up the diets. With respect to current species being cultured at IMTA sites, the settling velocity of mussel feces are quite low when compared with those reported for salmon feces ( $3.2 \pm 1.1$  cm/sec) and salmon feed (6–15 cm/sec) (Chen et al. 1999a, Chen et al. 1999b, Cromey et al. 2002).

Several authors have gathered empirical data on density of feces, whereas others have attempted to use Stokes' law and Newton's second law to calculate and model settling velocities from inputs dictated by Komar et al. (1981) (Taghon et al. 1984, Elberizon & Kelly 1998, Chen et al. 2003, Magill et al. 2006). We found that mussel feces did not meet the assumptions of Reynolds number and thus Stokes' law could not be used to calculate settling velocities of mussel feces (not shown). Furthermore, mussel feces also do not satisfy the assumption of spherical shape dictated by Stokes' law. Komar et al. (1981) modified assumptions of shape in Stokes' law, and were able to report a good association with settling velocity of copepod fecal pellets. A recent review on the properties of salmon feces found no literature that applies these modifications to any other species (Reid et al. 2009). Because of the poor correlations observed with using Stokes' law, we deemed it necessary to collect empirical data on morphology and settling velocity of feces from various mussel size classes to fulfill IMTA modeling requirements.

#### Dispersion and Ecological Implications

The suspension culture of blue mussels operates on the basis of a predetermined density of mussels attached to a core substrate hung at an appropriate depth for the specific culture area. To determine the dispersion potential of mussel feces at any culture location, the following information is required: feces settling velocities, culture depth, and current velocities. For example, pooled diet settling velocity data from the current study (0.18, 0.29, and 0.54 cm/sec of 50% of particles for small, medium, and large mussels, respectively) at an arbitrary Quoddy region culture depth of 20 m and a current velocity of 15 cm/sec can be used to calculate "time to bottom." The deposition of feces according to these conditions would create a 1,660-, 1,111-, and 588-m-wide footprint for the respective size classes. The mussel size classes examined in the current study compare with industry standards for socking conditions, whereas harvesting sizes are slightly smaller. These dispersal predictions give significantly larger footprint estimates compared with footprints of 31 m and 124 m for large mussels (*P. canaliculus*) fed natural and monoculture algal diets, respectively, (Giles & Pilditch 2004), and footprints of 1.0–3.5 m and 7.0–24.4 m for mussel of shell length 3.0–4.5 cm and 5.5–7.5 cm, respectively (Callier et al. 2006). Note that both of these previous studies reported similar settling velocities of mussel biodeposits. The variations in dispersal distance appear to be more of a function of current velocity and depth at the site rather than settling velocities of feces, with small variations in the previously mentioned parameters causing significant impacts on dispersion (Giles & Pilditch 2004). Therefore, deposition will be concentrated closer to operations in culture environments where shallow water depth and low current velocities are predominant site characteristics. Within these described environments, studies have suggested that benthic sediment chemistry and communities are significantly affected by high levels of mussel biodeposition resulting from poor flushing characteristics (Dahlback & Gunnarsson



1981, Christensen et al. 2003, Hartstein & Stevens 2005, Callier et al. 2006, Cranford et al. 2007). When the biophysical properties of mussel feces and salmon wastes (feces and feed) are compared, it can be concluded that theoretical effects associated with mussel feces will be less than effects associated with salmon farm wastes.

Differences in settling characteristics and organic composition of mussel feces, salmon feces, and salmon feed have interesting implications with respect to managing benthic impacts. The current study shows that mussel feces have lower settling rates and OC compared with salmon feces and feed (0.2–1.6 cm/sec vs. 3.2–10 cm/sec and ~20–50% OC vs. ~75–90% OC, respectively), thereby suggesting significant differences in loading potentials for each species. Mirto et al. (2000) reported a comparison of microbial and meiofaunal responses to both intensive mussel farming and fish farming in the western Mediterranean, and reported that mussel farms decreased benthic indexes by only 25%, compared with a 50% change in benthic indexes under a nearby fish farm. Our findings seem to be consistent with impacts reported by Mirto et al. (2000).

The premise of the IMTA concept is to have wastes produced by upper trophic species consumed by lower trophic species cultured using different techniques around the main cages where the fish are fed. Ideally, cocultured extractive species will have the ability to absorb high proportions of waste particles produced by the upper trophic species, thereby reducing the overall load of organic material on the bottom. Although the current study confirms that mussels will absorb some salmon farm wastes efficiently, we did not determine whether the amount of feces produced over time differed between mussels fed algal- or salmon farm-based diets. We also recognize that mussels will also contribute to the deposition on the bottom, and are only partially efficient at removing wastes. In addition, as mussels grow, their impact on the bottom near the fish farm through the OC of their feces will likely become more prominent from a near-field perspective, because the settling velocity of the feces increases with mussel size.

There are wastes that are indigestible to mussels, and particles too large for ingestion, suggesting that loading under IMTA cages could benefit from further integration of some

detritivore species. The proposed approach to deal with mussel deposition in the IMTA strategy is to add additional extractive species, such as sea urchins (*Strongylocentrotus droebachiensis*) and sea cucumbers (*Cucumaria frondosa*), underneath mussel cages that can further strip organic matter produced by the upper trophic species and integrated mussels combined.

## SUMMARY

Particulate by-products of salmon culture were found to have minimal effect on the biophysical properties of mussel (*Mytilus* spp.) feces when compared with feces produced on algal-based diets. Differences in mussel feces width were found to be minimal in the small and medium mussels, but became more significant as mussel shell length increased (45–74 mm). Mussels have the capability of removing some of the organic component from salmon farm waste particles with relatively high efficiency. Regardless of diet, smaller mussels produce feces that disperse as a function of settling velocity (50% of the particles; small, 0.18 cm/sec; medium, 0.29 cm/sec) over larger areas than those produced by larger mussels (0.54 cm/sec). This study demonstrates the likelihood of the depositional footprint decreasing in size with increasing size of the mussels. The extent of the footprint will also be dependent on the hydrodynamics of the area and the culture depth. This study suggests that further field-based research is required on the loading potential of commercial biomass of mussel densities in an active IMTA farm. It would also be beneficial to determine whether higher concentrations of inorganic silt particles in suspended particulate matter will change significantly the settling velocity of feces produced between natural conditions and an IMTA environment.

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